THE PARTITIONING OF BODY WATER IN OSTEICHTHYES: PHYLOGENETIC AND ECOLOGICAL IMPLICATIONS IN AQUATIC VERTEBRATES[1]

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Great strides have been made in recent years in understanding the fluid medium in which metabolic processes occur. Investigations have been largely confined to qualitative studies of the constituents of the body fluids and to the relationship of qualitative shifts to water balance or imbalance. Although water itself is the fundamental substance involved in fluid balance, it is a curious fact that, until recently, there had apparently been no attempt made to determine the apportionment of the total body water among the fluid compartments of vertebrates, except in man and a few other mammals.

Reichert and Brown (1909) and more recently Florkin (1949), among others, have ascribed both phylogenetic and ecological significance to qualitative features of body fluids. It was thought that a comparative, quantitative study of vertebrate fluid compartments might demonstrate patterns which would likewise have phylogenetic and/or ecological implications. Data have been published on the Chondrichthyes (Thorson, 1958) and on Agnatha (Thorson, 1959), and this paper presents findings on the third aquatic vertebrate class, Osteichthyes, the bony fishes.

Only six papers known to the writer have previously reported measurements of blood volume in bony fishes. These are listed in Table III, which will be treated later. Only Prosser and Weinstein (1950) have attempted measurement of the extracellular fluid volume (NaSCN space) of fish, in a single species; but no attempt has previously been made at a complete quantitative analysis of the major fluid compartments of Osteichthyes.

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TABLE I

Summary of experimental data. The mean is followed by the number of experimental animals (in parentheses). The range of measurements appears in parentheses under the mean. Standard deviation is given for plasma, extracellular fluid and total body water

	Fresh-water Chondrostei		Fresh-water Holostei		Fresh-water Teleostei		
	Lake sturgeon	Paddlefish	Bowfin	Shortnose gar	Common sucker	Carp	Bigmouth buffalofish
Weight (gm.)	3058 (8) (2275–4530)	4679 (5) (3740–5910)	1963 (6) (1020–3265)	1185 (7) (855–1730)	617 (2) (580–655)	2412 (7) (1585–3190)	3395 (8) (1980–5440)
Pulse (beats/min.)	49 (8) (44–52)	22 (5) (16–28)	20 (6) (14–28)	19 (6) (14–24)	55 (2) (47–64)	28 (6) (18–39)	38 (8) (19–70)
Respiration (per min.)	53 (8) (40–72)	17 (5) (10–26)	14 (6) (9–20)	28 (5) (24–36)	47 (2) (45–50)	27 (3) (19–38)	22 (4) (20–24)
Hematocrit (per cent cells)	22 (8) (19–29)	30 (5) (24–37)	32 (6) (32–34)	42 (7) (33–50)	39 (2) (39–40)	33 (7) (23–44)	29 (8) (18–40)
Spec. grav., plasma	1.016 (3) (all 1.016)	1.017 (3) (1.016–1.018)	1.018 (3) (1.0175–1.0185)	1.019 (3) (1.016–1.021)	1.016 (3) (1.015–1.017)	1.019 (3) (1.018–1.020)	1.016 (3) (all 1.016)
Spec. grav., blood	1.036 (3) (1.033–1.040)	1.040 (3) (1.039–1.041)	1.045 (3) (1.044–1.047)	1.051 (3) (1.050–1.052)	1.041 (3) (1.040–1.042)	1.040 (3) (1.039–1.0405)	1.042 (3) (1.041–1.043)
Plasma volume (T-1824 space)*	2.8 (8) (2.2–3.9) s.d., .50	2.2 (5) (1.8–2.3) s.d., .21	2.2 (6) (1.9–3.2) s.d., .46	2.1 (7) (1.6–2.6) s.d., .38	1.2 (2) (1.0–1.5) s.d., .25	1.8 (7) (1.4–2.2) s.d., .27	1.9 (8) (1.3–3.3) s.d., .61
Blood volume*	3.7 (8) (2.8–4.9)	3.0 (5) (2.4–3.6)	3.4 (6) (2.9–5.0)	3.8 (7) (3.0–5.2)	2.2 (2) (1.8–2.7)	3.0 (7) (2.4–3.5)	2.8 (8) (1.8–4.1)
Extracellular fluid (sucrose space)*	20.1 (8) (15.4–27.5) s.d., 3.91	15.6 (5) (14.1–16.9) s.d., .95	18.9 (6) (14.6–25.6) s.d., 3.31	13.6 (7) (12.5–14.8) s.d., .86	12.2 (2) (11.8–12.7) s.d., .45	15.5 (7) (12.0–22.1) s.d., 3.34	13.2 (8) (10.9–15.6) s.d., 1.27
Interstitial fluid (sucrose space minus plasma)*	17.3	13.4	16.7	11.5	11.0	13.7	11.3
Total body water*	72.7 (8) (71.5–74.0) s.d., .70	74.0 (5) (71.3–77.4) s.d., 2.07	74.5 (6) (72.6–76.4) s.d., 1.18	66.7 (7) (64.5–70.7) s.d., 1.85	74.4 (2) (74.1–74.8) s.d., .35	71.4 (7) (70.1–73.1) s.d., 1.19	70.6 (8) (64.1–76.0) s.d., 3.45
Intracellular fluid (total water minus sucrose space)*	52.6	58.4	55.6	53.1	62.2	55.9	57.4

* All fluid volumes expressed as per cent of body weight.

TABLE I—*Continued*

	Marine Teleostei						
	Tiger rockfish	Nassau grouper	Red snapper	Gray snapper	Green moray	Great barracuda	Rainbow parrotfish
Weight (gm.)	5885 (1)	1270 (2) (930–1610)	3765 (2) (3130–4400)	3711 (6) (1900–4680)	4062 (6) (3050–4815)	2204 (11) (1430–4575)	4607 (19) (1650–6830)
Pulse (beats/min.)	51 (1)	52 (1)	58 (2) (48–68)	54 (3) (44–66)	70 (5) (60–88)	68 (11) (32–98)	48 (16) (30–98)
Respiration (per min.)	—	—	—	41 (4) (30–48)	22 (3) (18–28)	41 (7) (30–54)	40 (14) (22–64)
Hematocrit (per cent cells)	28 (1)	28 (2) (28–29)	36 (2)	35 (6) (28–40)	26 (6) (24–28)	31 (11) (25–36)	30 (27) (20–40)
Spec. grav., plasma	Used ave. (1.017)	Used ave. (1.017)	Used ave. (1.017)	Used ave. (1.017)	Used ave. (1.017)	Used ave. (1.017)	Used ave. (1.017)
Spec. grav., blood	Used ave. (1.042)	Used ave. (1.042)	Used ave. (1.042)	Used ave. (1.042)	Used ave. (1.042)	Used ave. (1.042)	Used ave. (1.042)
Plasma volume (T-1824 space)*	2.3 (1)	1.8 (2) (1.6–2.1) s.d., .25	1.3 (2) (1.3–1.4) s.d., .05	1.3 (6) (1.2–1.5) s.d., .10	1.6 (6) (0.7–2.3) s.d., .63	1.9 (10) (1.5–2.6) s.d., .30	2.4 (16) (1.9–3.2) s.d., .42
Blood volume*	3.3 (1)	2.6 (2) (2.3–3.0)	2.2 (2)	2.0 (6) (1.9–2.5)	2.2 (6) (1.0–3.0)	2.8 (10) (2.3–3.7)	3.6 (16) (2.5–4.7)
Extracellular fluid (sucrose space)*	12.5 (1)	14.5 (2) (13.1–16.0) s.d., 1.45	14.0 (2)	14.0 (6) (12.2–15.3) s.d., .97	15.8 (6) (12.2–19.4) s.d., 2.67	15.9 (8) (11.6–19.1) s.d., 2.07	16.6 (8) (14.3–18.9) s.d., 1.60
Interstitial fluid (sucrose space minus plasma)*	10.2	12.7	12.7	12.7	14.2	14.0	14.2
Total body water*	71.1 (1)	71.7 (2) (70.4–73.1) s.d., 1.35	71.3 (2) (71.2–71.5) s.d., .15	72.3 (6) (70.5–74.0) s.d., 1.02	63.7 (6) (56.1–71.8) s.d., 5.92	70.6 (9) (68.1–73.3) s.d., 1.94	73.1 (14) (71.2–75.0) s.d., 1.20
Intracellular fluid (total water minus sucrose space)*	58.6	57.2	57.3	58.3	47.9	54.7	56.5

TABLE II

Experimental data summarized by taxonomic groups and compared with data for Agnatha and Chondrichthyes. Mean is followed by number of experimental animals in parentheses. Standard deviation given for plasma, extracellular fluid and total body water. Ranges can be determined from Table I

	Agnatha (*Petromyzon marinus*)	Chondrichthyes (summary)	Osteichthyes (summary)	Fresh-water Chondrostei	Fresh-water Holostei	Fresh-water Teleostei	Marine Teleostei
Weight (gm.)	190 (12)	3012 (65)	3195 (90)	3681 (13)	1544 (13)	2664 (17)	3710 (47)
Pulse (per min.)	31 (12)	24.5 (39)	44.5 (80)	39 (13)	20 (12)	37 (16)	57 (39)
Respiration (per min.)	122 (12)	—	35.3 (61)	47 (13)	20 (11)	29 (9)	38 (28)
Hematocrit (per cent cells)	33 (12)	18.3 (52)	30.7 (98)	25 (13)	38 (13)	32 (17)	30 (55)
Spec. grav., plasma	1.018 (12)	—	1.017 (21)	1.0165 (6)	1.0185 (6)	1.017 (9)	—
Spec. grav., blood	1.040 (12)	—	1.042 (21)	1.038 (6)	1.048 (6)	1.041 (9)	—
Plasma volume (T-1824 space)*	5.5 (12) s.d., .32	5.4 (44) s.d., 1.3	2.0 (86) s.d., .41	2.5 (13) s.d., .41	2.1 (13) s.d., .42	1.8 (17) s.d., .46	1.9 (43) s.d., .38
Blood volume*	8.5 (12)	6.6 (44)	3.0 (86)	3.5 (13)	3.6 (13)	2.8 (17)	2.9 (43)
Extracellular fluid (sucrose space)*	23.9 (12) s.d., .79	21.2 (3) s.d., 2.38	15.7 (76) s.d., 2.28	18.4 (13) s.d., 3.12	16.0 (13) s.d., 2.34	14.0 (17) s.d., 2.31	15.4 (33) s.d., 1.80
Interstitial fluid (sucrose space minus plasma)*	18.4 (12)	15.8	13.7	15.9	13.9	12.2	13.5
Total body water*	75.6 (12) s.d., .51	74.8 (42) s.d., 1.6	71.2 (83) s.d., 2.29	73.2 (13) s.d., 1.40	70.3 (13) s.d., 1.58	71.4 (17) s.d., 2.47	70.8 (40) s.d., 2.62
Intracellular fluid (total water minus sucrose space)*	51.7	53.6	55.5	54.8	54.3	57.4	55.4

* All fluid volumes expressed as per cent of body weight.

TABLE III

Blood volumes of Osteichthyes reported in the literature

Author	Species	Method	No. of specimens	Per cent body weight
Welcker (1858)	*Cyprinus tinea*	Direct (Hb)	1	1.87
Welcker (1858)	*Perca fluviatilis*	Direct (Hb)	1	1.07
Derrickson and Amberson (1934)	*Tautoga onitis*	Direct (Hb)	3	1.5
Prosser and Weinstein (1950)	*Ictalurus natalis*	T-1824	6	1.77
Martin (1950)	*Ophiodon elongatus*	T-1824	8	2.8
Martin (1950)	*Ophiodon elongatus*	Vital Red	1	1.9
Martin (1950)	*Sebastodes sp.*	Vital Red	1	2.8
Martin (1950)	Cottidae (sculpin)	Vital Red	3	2.3
Lennon (1954)	*Catostomus commersoni*	Tail severance (bleeding)	23	1.5
Schiffman and Fromm (1959)	*Salmo gairdneri*	T-1824	10	2.25

Commission; James T. Shields, Superintendent of Fisheries, South Dakota Department of Game, Fish and Parks; E. C. Saeugling, U. S. Fish and Wildlife Service Hatchery Manager, Guttenberg, Iowa; K. H. Loftus, Head, Fisheries Section, and W. J. Christie, Biologist, Division of Research, Ontario Department of Lands and Forests.

Materials and Methods

Of the marine fish employed (all Teleostei), the great barracuda, *Sphyraena barracuda,* rainbow parrotfish, *Pseudoscarus guacamaia,* tiger rockfish, *Mycteroperca tigris,* and one gray snapper, *Lutianus griseus,* were collected from the waters surrounding the islands of Bermuda. The remainder of the gray snappers and the red snappers, *Lutianus campechanus,* were taken off the coast of Florida in the general vicinity of St. Augustine. The Nassau grouper, *Epinephelus striatus,* and green moray, *Gymnothorax funebris,* were collected off the Florida coast near the Miami area.

Of the fresh-water fish, all the teleosts were taken from Nebraska lakes or streams: bigmouth buffalofish, *Ictiobus cyprinellus,* carp, *Cyprinus carpio,* and common white sucker, *Catostomus commersoni.* Of the Holostei (fresh-water forms), the shortnose gar, *Lepisosteus platostomum,* were taken in eastern Nebraska, and the bowfin, *Amia calva,* from northeastern Iowa. Of the fresh-water Chondrostei, the paddlefish, *Polyodon spathula,* were from eastern Nebraska, and the lake sturgeon, *Acipenser fulvescens,* from the St. Lawrence River in southern Ontario.

All fish were taken in nets or traps, so were undamaged, except the lake sturgeon, which were hooked by commercial fishermen and held in a large tank for several weeks before they were used.

The fish were anesthetized with approximately 50 mg./kg. aqueous pentobarbital sodium (Nembutal) injected intraperitoneally. Respiratory movements usually continued, but the gills were irrigated artificially throughout the experiments. The anesthetic required about 30–60 minutes to take full effect, and anesthesia lasted for several hours.

Plasma and extracellular fluid volumes were measured by dilution methods modified from Keith, Rowntree and Geraghty (1915). T-1824 (Evans Blue) was employed for the former, and sucrose for the latter.

After a fish was anesthetized, a cardiac puncture was made and blood was drawn into heparinized capillary tubes for hematocrit determination. A measured quantity of blood was then drawn into aqueous potassium oxalate. The syringe was doubly calibrated to receive nine parts of blood and one part oxalate. This blood was centrifuged and the plasma was used in the blank and standard dilutions employed in colorimetry, as a control on substances normally present in plasma. A solution containing 25 mg. % T-1824 and 3% sucrose was next injected into the heart in a quantity equal to that of the blood drawn. After mixing had occurred, samples of blood were withdrawn from the heart for colorimetric determination of the dilution of the injected materials. By comparison with known dilutions, the volume occupied by the dye or sucrose could readily be calculated. However, since both T-1824 and sucrose disappear slowly from the blood, a volume calculated at one instant will be smaller than one calculated later. To obtain the theoreti-

cal concentration with complete mixing but no loss from the system, several blood samples were withdrawn over a period of time and the optical density of T-1824 and sucrose extrapolated to the time of injection (Erlanger, 1921). Equilibration of the dye was rapid, and samples were drawn at 5–10-minute intervals for about 40 minutes. Extrapolation curves showed that sucrose required about an hour to be distributed thoroughly, so less frequent samples were drawn over a period of 3–5 hours for extracellular fluid measurements. All plasma for blank, standard dilutions, and tests samples was diluted 1 : 10 with isotonic saline.

Sucrose analysis was by a modification of the method of Harrison (1942). To remove the dye complexed with plasma proteins, the plasma was deproteinized by the zinc sulphate method of Somogyi (1930). Blood glucose, which would interfere with the analysis, was removed by Little's (1949) method of heating the plasma with sodium hydroxide. Color was developed by the standard diphenylamine method, and optical density was determined with a Bausch and Lomb Spectronic 20 colorimeter at 620 mμ, as it was also for T-1824.

Whole blood volume was calculated from plasma volume and hematocrit, and total body water was determined by complete desiccation of the whole animal at 105° C.

For conversion of volume measurements to per cent of body weight, specific gravity of whole blood and plasma were required. These were determined by the method described by Todd and Sanford (1943), in which a mixture of two miscible substances (chloroform, s.g. 1.489, and benzene, s.g. .879) was adjusted to suspend a drop of the test substance. The specific gravity of the mixture was then determined by a small hydrometer, suitably calibrated and corrected for temperature.

Discussion of Results

A summary of data, by species, is presented in Table I. Each mean figure is followed in parentheses by the number of experimental animals employed. Below the mean is the range of measurements. For plasma volume, sucrose space, and total body water, the three basic fluid measurements, the standard deviation is also given. All fluid compartment measurements are expressed as percentages of body weight.

The figures for species in which only one or two animals were used cannot be regarded as reliable in view of the individual variations evident in the tabulation of ranges. Nevertheless, these have been included in the table and have been used for group comparisons in Table II. In the latter table, species have been summarized into four groups, to make possible comparisons based on habitat and major taxonomic groups. To these have been added data from previous work by the author on the sea lamprey, *Petromyzon marinus*, an agnathan (Thorson, 1959), and four species of Chondrichthyes: *Squalus acanthias*, the spiny dogfish; *Raja binoculata*, the big skate; *Raja rhina*, the long-nosed skate; and *Hydrolagus collici*, the rat-fish or chimaera (Thorson, 1958).

The paddlefish and sturgeon are members of the Chondrostei, which have a prominent, persistent notochord, a cartilaginous skeleton, and a heterocercal tail. They are generally considered the most primitive of living ray-finned fishes and, of all the species used, most closely allied to the ancestral stock. The bowfin and gar belong to the Holostei, which in general exhibit a less prominent notochord, a

partially ossified skeleton, and an abbreviated heterocercal tail. They are thought to have arisen from the chondrostean line and are more advanced than the latter, but primitive in relation to the teleosts. The Teleostei include the most advanced of the fishes and are now the dominant form of life in most waters of the earth. They have a largely ossified skeleton, the least persistent notochord, and usually a homocercal tail. They are represented in this work by ten species, the buffalofish, sucker and carp from fresh water, and the remainder from salt water. Only teleosts have been used for comparison of fresh-water with salt-water forms.

Weight

Ideally, when species are compared, animals of comparable size should be used. For practical reasons concerning supply of animals, this was not possible. Martin (1950), working with *Squalus acanthias,* reported a greater blood volume in small fish than in large ones of the same species. If this phenomenon is of general application, comparative size would be a matter of great concern. Although some series of present measurements suggested the same findings as Martin's, the pattern was not consistent enough to warrant a positive statement. Species have therefore been compared regardless of size, although reservations on these grounds may be in order.

Respiratory movements

The rates of respiratory movements were determined after anesthesia and cannot be assumed to reflect accurately the respiratory activities in unanesthetized animals. Considerable diversity exists, figures ranging from 14 per minute in the bowfin to 53 in the lake sturgeon. Great diversity is evident within each of the four major groups, and no consistent pattern is discernible which can be related to taxonomic categories. Three of the primitive species have among the lowest respiratory rates (gar, bowfin and paddlefish), but the sturgeon tends to negate the conclusion that a slow respiratory rate is a primitive characteristic. The average rate of all fresh-water species, regardless of taxonomic group, is about 33, as compared with 38 for marine species. It is tempting to speculate on the ecological significance, but actually this is not a great difference in view of the great diversity within both groups. It would seem reasonable that the rate of respiratory movements would be related to metabolism, but figures are not available on metabolic rates of these species. The possibility also suggests itself that a correlation might exist between respiratory rate and general visible activity. No quantitative study was made, but general observations do not support such conclusions. It is true that the paddlefish (17 per minute) is relatively sluggish. After removal from water it scarcely struggled, and could probably be worked on without anesthesia, although this was not done. The bowfin, however (14 per minute), was far from sluggish and docile, and the sturgeon (53 per minute), although not as sluggish as the paddlefish, was far less active than most of the other species.

Pulse

The pulse rates were also taken immediately after anesthesia, so cannot with confidence be compared with rates of unanesthetized animals. The most striking

observation from the present measurements is the rather close correspondence of relative pulse rate with the rate of respiratory movements. With only minor discrepancies, a relatively high rate of heart beat is found in species with a high respiratory rate. This is also apparent when the four summarized groups are compared. However, in this case, there is an appreciable difference between the average pulse rate of marine teleosts (57 per minute) and fresh-water teleosts (37) or the average of all fresh-water species of Osteichthyes, including the primitive one (32). The higher pulse rate, perhaps together with the somewhat higher rate of respiratory movements of marine fish, is very likely related to the difference in external medium and/or to basal metabolism and its visible manifestation, relative bodily activity. These data point up strongly the need for work on metabolic rates in these species. Since the gar, bowfin and paddlefish have decidedly the lowest pulse rates of all species studied, a strong case could be made for slow pulse as a primitive characteristic, but for the sturgeon, whose pulse rate of 49 is in the range of teleost rates. Even so, the average of all the Chondrostei and Holostei is 29 (fresh-water teleosts 37, marine teleosts 57) so it might be said that a slow pulse is a tendency in the primitive fishes, even though the lake sturgeon is an exception.

Specific gravity of plasma and whole blood

Specific gravities were determined only for fresh-water species, so comparisons cannot be made between species of different external medium. No correlation is evident between specific gravity of whole blood and phylogenetic position of the three groups of fresh-water fish. The differences between plasma specific gravity values for the three groups are so small as to be virtually meaningless. No published plasma values were available for marine fish. Martin's (1950) average figure for whole blood of three ling cod (*Ophiodon elongatus*) was 1.044. For purposes of calculations in the marine species, the mean values obtained for fresh-water fish were used (1.017 for plasma; 1.042 for whole blood).

Hematocrit

The per cent of cells in the blood was determined in each animal for calculation of whole blood from plasma volume. The results are in general agreement with figures given by Martin (1950) and Prosser and Weinstein (1950), although they are far above the 7% figure given by Welcker (1858), which must have been in error. There is little difference between the hematocrit of marine teleosts (30) and fresh-water teleosts (32) or all fresh-water species (31). There is no clear correlation with the phylogenetic sequence, nor with differences in pulse rate or respiratory movements. However, the differences in hematocrit of the three groups of fresh-water species correspond, in a manner that could be expected, with the differences in specific gravity of whole blood.

Plasma and whole blood volume

Techniques of plasma and blood volume determination have been debated vigorously for many years and have been discussed most recently by Gregersen and Rawson (1959). It is recognized that the T-1824 dilution method has certain limitations, but still more serious objections have been raised to many

other methods. In light of published discussions, as well as for practical considerations, T-1824 was selected for earlier work by the writer (1958, 1959). For the purpose of reliable comparison, it has been used in this investigation and will continue to be used in the other vertebrate groups.

When a dye is introduced into the bloodstream it is distributed only in the fluid portion of the blood, and therefore calculation of the volume it occupies is a measure of the plasma rather than whole blood. Confusion on this point (see discussion in Gregersen and Rawson, 1959) by some early users of the dilution method resulted in some plasma volumes being published as blood volumes. As a part of a study of partitioning of body fluids, plasma volume is the pertinent measurement since the water in the corpuscles is not a part of the extracellular or vascular water, but is rather a part of the intracellular compartment. However, from the standpoint of the heart and general circulation, the whole blood volume is the important consideration. Measurements of both volumes are included here.

Plasma and blood volumes of bony fishes have long been known to be low in comparison with those of other vertebrates, but recorded measurements have been rather fragmentary. All known previous measurements concerned exclusively with Osteichthyes are included in Table III. Most of the earlier results are lower than mine. However, in the most extensive study (Martin, 1950), all individual figures were within the range of variation of individual teleosts in the present study (Table I), and the average figure (2.6% of body weight) of Martin's teleosts (all marine) was only slightly lower than my marine teleosts (2.9%) shown in Table II.

It was suggested by the writer (1959) that, among aquatic vertebrate classes, a relatively large plasma and blood volume might be regarded as a primitive characteristic. *Petromyzon marinus* (Agnatha, the most primitive class of vertebrates) has a plasma volume of 5.5% of the body weight, and blood volume of 8.5% (Table II). Chondrichthyes, also a relatively primitive class, but more advanced than agnathans, has virtually the same plasma volume (5.4%), but, in agreement with the lower hematocrit value, has a lower blood volume (6.6%). None of the species of Osteichthyes, the most advanced of the three aquatic vertebrate classes, had a plasma volume of more than 2.8% (lake sturgeon) or a blood volume of more than 3.8% (shortnose gar). The case for the phylogenetic significance of vascular volume is supported by small differences in plasma volume in the three groups of fresh-water Osteichthyes. The Chondrostei, the most primitive, averaged 2.5%, the intermediate Holostei 2.1%, and the most advanced Teleostei 1.8%. The same is evident for blood volumes except that the higher hematocrit value of the Holostei increases their blood volume (3.6%) to slightly more than that of the Chondrostei (3.5%). All four of the primitive species consistently equal or exceed all species of teleosts in plasma and blood volume, except the tiger rockfish, whose single specimen is by itself virtually meaningless, and the rainbow parrotfish. A satisfactory explanation of the latter species' relatively high plasma volume (2.4%) and blood volume (3.6%) has not been found. Sufficient specimens (16) were employed to obtain reliable results; the species cannot be considered as primitive among the teleosts on morphological grounds; its external medium is not different from that of the other marine species; comparative metabolic rates are not known, and relative activity

was not determined; and Martin's (1950) size : blood volume relationship cannot account for the "discrepancy," since these fish were among the largest employed.

A comparison of fresh-water teleosts with marine teleosts reveals a striking similarity in both plasma and blood volumes, only 0.1% separating the two groups in both cases. Considering the radically different osmotic environment of the two groups and the great divergence in physiology of water balance, this is a remarkable example of phylogenetic homeostasis.

The difference between the two groups is so slight as perhaps to be meaningless, but the fact that the figures for the marine species are higher than those for fresh-water species should be kept in mind when the sucrose space is considered later.

Extracellular fluid volume (sucrose space)

The closest approximation to the extracellular fluid volume attainable with available methods is the calculation of the volume occupied by a substance which, when injected into the blood stream, will filter readily from the capillaries into the intercellular spaces. The substance must not be metabolized or excreted

TABLE IV

Comparison of inulin, raffinose and sucrose spaces of Pseudoscarus guacamaia

	Vol. (per cent body wt.)	Range	Aver. wt. (grams)	No. of specimens
Inulin	11.4	9.2–14.5	5451	8
Raffinose	14.4	12.7–16.4	4096	4
Sucrose	16.6	14.3–18.9	4696	8

rapidly, and must not be extensively taken up by the cells. It should also penetrate the minor fluid compartments, such as coelomic, cerebrospinal, and ocular fluids, which are likewise extracellular. A substance which satisfies all of these demands has not yet been found, but a number of compounds have been used which appear to approach the specifications in varying degree. Among them are thiocyanates, ferrocyanides, sulfates, chlorides, and bromides, and various carbohydrates such as inulin, raffinose, and sucrose. Inorganic ions are unsuitable for extracellular fluid determinations of marine fishes, since they are excreted rapidly by the gills (Smith, 1930). This was also demonstrated with thiocyanate in marine Chondrichthyes (Thorson, 1958), so such substances were not employed on the Osteichthyes.

In my work on Chondrichthyes, the spaces penetrated by inulin, raffinose, and sucrose were compared. These were, in *Squalus acanthias*, respectively, 12.7, 15.2, and 21.2% of the body weight. These volumes are in inverse order to the molecular weights of the three substances: 990, 594, and 342. So that a comparison with at least one bony fish could be made, the same three substances were also compared in the rainbow parrotfish (Table IV), and much the same results were apparent. Sucrose appeared to penetrate more thoroughly in both groups of fish than either inulin or raffinose. Sucrose is considered by many investigators as the substance of choice for higher vertebrates (Robertson, 1953; Wilde, 1945;

Kruhøffer, 1946). It was used in this study, and for purposes of comparison will be employed in future studies on other vertebrate groups.

None of the substances employed by myself and other workers has been shown to penetrate appreciably into the minor fluid compartments. In Chondrichthyes (Thorson, 1958) coelomic, cerebrospinal, and ocular fluids were analyzed for both inulin and sucrose penetration, and the results were negative. In the present work, only the ocular fluid was of sufficient quantity to test, and sucrose was not found in it. This introduces a small error into the extracellular fluid measurements, but the cerebrospinal and coelomic fluids were so small in quantity that they could not be measured directly and it is estimated that the ocular fluid would not make up more than about one-fourth of one per cent of the body weight in any of the species employed. No correction for minor fluids has been introduced in the tables.

It should be borne in mind that the fluid reached by the sucrose includes also lymph, since this is interstitial fluid en route back into the vascular system. It has been impossible to measure lymph separately, so it is included with the interstitial fluid volume.

When the sucrose spaces of the three fresh-water groups are compared, an even more pronounced relationship in the taxonomic series appears than for plasma volume (Table II). The more primitive the group, the higher is its extracellular fluid volume: Teleostei, 14.0%; Holostei, 16.0%; Chondrostei, 18.4%. This continues to be apparent when the comparison is extended to the Chondrichthyes (21.2%) and Agnatha (*Petromyzon marinus*, 23.9%), the most primitive class having the largest sucrose space of all the aquatic classes.

It must be noted that the figure for extracellular fluid volume of the Chondrichthyes is based on only three individual measurements. These are three *Squalus acanthias*, the only chondrichthyans in which sucrose was employed. Otherwise, all extracellular fluid measurements of the four species of Chondrichthyes were made with the use of inulin, which gave lower results (see above). The average inulin space of 31 chondrichthyan fish was 11.9% of the body weight. This figure can be compared directly only with the rainbow parrotfish, in which the inulin space was 11.4% (Table IV). If the sucrose space of the rainbow parrotfish (16.6%, based on eight individuals) is increased by a factor of 11.9/11.4, the result might be used as a calculated sucrose space for Chondrichthyes. This would be 17.3%, as compared with 15.7 for Osteichthyes and 23.9 for *Petromyzon marinus*. The same gradient in extracellular fluid volume is apparent as before, although the difference between Chondrichthyes and Osteichthyes is not as pronounced.

When fresh-water teleosts are compared with marine teleosts, it is seen that the latter have a somewhat larger sucrose space (15.4%) than the former (14.0%). Considering the extreme osmotic dissimilarity of the fresh- and salt-water media, these figures may be more remarkable for their relative closeness than for the slight difference apparent. The difference which exists is probably related, among other things, to the slightly higher osmotic pressure of the blood of marine fish as compared with fresh-water fish (Prosser *et al.*, 1950; Florkin, 1949; Williams, 1951).

There is some variation within all the groups, and, as for plasma and blood

volumes, the rainbow parrotfish has the largest sucrose space of all the marine teleosts, although the difference is not as great in this case. Its sucrose space is also greater than that of any fresh-water teleost and only that of the bowfin and rock sturgeon of the primitive forms exceeds it.

Only one earlier measurement of the extracellular fluid compartment in any aquatic vertebrate is known to the writer. Prosser and Weinstein (1950) determined the thiocyanate space of six yellow bullheads, *Ictalurus natalis,* which are fresh-water teleosts. The average volume was 4.0% of the body weight. In this paper, the average for all fresh-water teleosts was 14.0. The lowest species figure was for the two common suckers (12.2), and the lowest individual measurement was 10.9 for a bigmouth buffalofish. No marine teleost figure was this low, although when inulin was employed (Table IV) a figure of 9.2 was obtained for a single rainbow parrotfish. In view of the great divergence in results, it is inconceivable that the measurements of Prosser and Weinstein could represent chance variation, and it appears probable that the explanation must be sought in differences in substances employed, technique, and interpretation of extrapolation curves.

Interstitial fluid

When the extracellular fluid volume (sucrose space) and plasma volume (T-1824 space) are known, a reasonable approximation of the interstitial (tissue) fluid volume can be reached by subtracting the former from the latter. The lack of information on the minor fluid compartments does not affect this calculation, since the sucrose does not penetrate these compartments. It should be remembered, however, that the figures for interstitial fluid (Tables I and II) include the lymph.

The interstitial fluid volume could not be calculated for every animal employed, since the extracellular fluid and plasma measurements were not always both successful in the same animal. For this reason, the interstitial fluid volume for every species or other taxonomic group was derived from the mean extracellular fluid and plasma figures for that species or group. Therefore, ranges and standard deviations are not given for interstitial fluid in Tables I and II.

The most primitive group (Chondrostei) has the largest interstitial volume (15.9% of the body weight), followed by the Holostei (13.9%); the fresh-water teleosts have the lowest volume (12.2%). The marine teleosts have a somewhat higher volume (13.5%) than the fresh-water teleost species. Comparing the three classes of aquatic vertebrates, Agnatha (*Petromyzon*) has the highest interstitial volume (18.4%), Chondrichthyes has a volume of 15.8%, and the Osteichthyes 13.7%. It is seen, then, that the volume of extracellular fluid relative to the taxonomic groups is reflected in a similar fashion in both of the extracellular sub-compartments, namely plasma and interstitial fluid.

The total circulating fluid, both intra- and extra-vascular, appears to be more plentiful in primitive aquatic vertebrates than in the more advanced, and slightly more plentiful in salt-water forms than in fresh-water.

Total body water

The use of antipyrine for determination of total body water (Soberman, 1950) was unsuccessful in marine Chondrichthyes (Thorson, 1958) and was not at-

tempted in the Osteichthyes. Complete dehydration at 105° C. was chosen as the method most practical for comparison of a wide variety of vertebrates.

In general, the total water content is fairly uniform among osteichthyan species, with two exceptions (Table I). The green moray contained only 63.7% water, appreciably below any other teleost species or the average for teleosts, either fresh-water or marine. This is almost undoubtedly due to the great quantity of oil contained in their bodies. The shortnose gar also had a lower water content (66.7%) than any of the fresh-water species. In this case, the low figure was probably related to the heavy investiture of ganoid scales.

The green moray and gar figures obscure what otherwise would be a comparative picture similar to, although less pronounced than, that for plasma and extracellular fluid. As it is, the fresh- and salt-water teleosts are very close in water content, marine forms having a little less rather than more. Holostei have somewhat less than fresh-water teleosts, even though the bowfin has the highest water content of all species measured. The most primitive group, Chondrostei, however, is true to form in having the highest content of all four groups. The Agnatha (*Petromyzon*) have the highest water content of all three classes (75.6%), as might be expected, and the Chondrichthyes (74.8) are also appreciably higher than the Osteichthyes (71.2).

Intracellular fluid

No method is known to the writer for direct measurement of the intracellular water of a whole organism, and no figures on volumes of the lower vertebrates are known from the literature. In the human the intracellular fluid compartment comprises about 50% of the body weight (Gamble, 1947). When total body water and extracellular water are known, the intracellular fluid volume can be calculated as the difference between the two. Since sucrose does not appear to become distributed in the minor fluids (coelomic, cerebrospinal and ocular), the intracellular fluid, calculated in this manner, includes the minor fluids as well. It has been pointed out that these are almost negligible, and the error thus introduced detracts little from the reliability of the results for comparative purposes.

As for interstitial fluid, the intracellular fluid volume for each group was derived from the mean figures for total water and extracellular water of that group, and ranges and standard deviations are not given in Tables I and II.

Variation between species in calculated intracellular fluid is considerable (Table I), and only when the summaries in Table II are considered is any pattern suggested. In a general way, the relationship of fluid volume to the taxonomic series is here almost exactly reversed from that evident for plasma and extracellular fluid. Of the three fresh-water groups, the two primitive ones have a smaller intracellular volume (Chondrostei, 54.8; Holostei, 54.3) than the teleosts (57.4). The marine teleosts have a smaller intracellular volume (55.4) than the fresh-water teleost species (57.4) and the two primitive classes (Agnatha, 51.7; Chondrichthyes, 53.6) have smaller volumes than the Osteichthyes (55.5).

It was seen in Table I that there was considerable difference between species in total water content, two species, especially, (green moray and shortnose gar) having a considerably lower water content than other species of their groups. This can probably be explained in terms of varying quantities of fats, scales, and

other solids in the bodies of the various species. However, the fact remains that differences in dry weight will affect the fluid measurements when expressed as per cents of total body weight. A more accurate picture of water partitioning can be had if volumes are expressed in terms of total water. Accordingly, in Table V, intra- and extracellular fluid compartments and the latter's sub-compartments, plasma and interstitial fluid, have been converted and expressed as per cents of total body water rather than per cents of body weight as before.

A comparison of these expressions of body fluid volumes points even more strongly than the observations made earlier to the existence of a relationship between body fluid partitioning in aquatic vertebrates and the phylogenetic series, as well as to habitat.

When the figures for extracellular fluid, plasma and interstitial fluid are arranged in order, from smallest to largest, the order of the groups is exactly the same: smallest for fresh-water teleosts, followed by marine teleosts, Holostei and

TABLE V

Body fluid measurements expressed as per cent of total water

	Agnatha (*Petromyzon marinus*)	Chondrichthyes (summary)	Osteichthyes (summary)	Osteichthyes			
				Chondrostei	Holostei	Fresh-water Teleostei	Marine Teleostei
Total water	100	100	100	100	100	100	100
Intracellular fluid	68.4	71.6	78.0	74.9	77.3	80.4	78.3
Extracellular fluid	31.6	28.3	22.0	25.1	22.7	19.6	21.7
Plasma	7.3	7.2	2.8	3.4	2.9	2.5	2.6
Interstitial fluid	24.3	21.1	19.2	21.7	19.8	17.1	19.1

Chondrostei, in that order. Thus, these volumes are highest in the most primitive Chondrostei and lowest in the most advanced teleosts (both fresh-water and marine). The order for intracellular fluid is exactly reversed, the greatest volume being found in the teleosts and the smallest in the primitive Chondrostei. When the comparison of all fluid volumes is extended from the relatively advanced class Osteichthyes to the more primitive Chondrichthyes, in every case the progression is in the same direction as for the groups within the Osteichthyes: larger extracellular, plasma and interstitial volumes; smaller intracellular. The progression is extended again when the most primitive of the three classes, Agnatha, is compared with the others.

It will be noted that the marine teleosts maintain their position in relation to fresh-water teleosts and the other groups in every fluid measurement: the extracellular fluid volume as well as its subdivisions, plasma and interstitial fluid, are slightly higher in marine than in fresh-water teleosts, but lower than those of any other group of aquatic vertebrates. The intracellular fluid is slightly less than that of fresh-water teleosts, but greater than that of any other group considered.

Some of the differences shown in the tables are small and statistically question-

able, and data for Agnatha are somewhat meager, being based on only one species. However, the over-all pattern which emerges appears highly suggestive. When comparisons progress in the direction of primitiveness in the whole series, or from fresh- to salt-water forms within the teleosts, the proportion of total water within the cells for use in the metabolic activities of the protoplasm appears to become less, and a greater proportion of water becomes available outside the cells for transportation of raw materials and metabolites between the cells and the external environment. The more advanced groups, and, concerning habitat, especially the fresh-water teleosts, appear to get along with a smaller amount of circulating fluid, which, then, is presumably used more effectively.

Summary

1. The major body fluid compartments were measured in two species of fresh-water Chondrostei, two species of fresh-water Holostei, three species of fresh-water Teleostei and seven species of marine teleosts. These were compared with previous measurements of an agnathan species and four species of Chondrichthyes.

2. A general correlation was shown between the relative rates of respiratory movements and pulse rates, but neither of these appeared to be related to the taxonomic series. A faster pulse was more characteristic of marine than of fresh-water species.

3. Plasma volume was measured by the dye dilution method, using T-1824. Whole blood volume was calculated from plasma volume and hematocrit. A progressive reduction in plasma and whole blood volume was noted, proceeding from the primitive to the more advanced groups. This is true both among the three classes of aquatic vertebrates and also among the three groups within the Osteichthyes. These volumes were remarkably similar in fresh-water and marine teleost species, although slightly higher in the latter.

4. Extracellular fluid volume was approximated by sucrose dilution. A comparison of inulin, raffinose and sucrose spaces in one species showed most thorough penetration by sucrose and least by inulin, the volumes being in inverse order to the molecular weights of the substances. Sucrose did not penetrate the minor fluid compartments (coelomic, cerebrospinal and ocular fluids) so these are not included in the extracellular data. The minor fluids probably do not exceed one fourth of one per cent of the body weight in any species studied. The sucrose space (extracellular fluid volume), like plasma volume, was found to be greatest in primitive forms and least in the most advanced. The fresh-water and marine forms have similar extracellular volumes, although slightly greater in the latter.

5. Interstitial (tissue) fluid was estimated by subtracting plasma from extracellular fluid. Exactly the same relationship with the taxonomic series and habitat obtained here as for plasma and extracellular fluid.

6. Total body water was measured by complete desiccation at 105° C. It was found to be fairly uniform among osteichthyan species, with two major exceptions, probably related to high oil content of the body and a heavy investiture of scales. The correlation of water content with the taxonomic series is not as clear in this case, although there is a greater water content in the Chondrostei than in the other two groups of Osteichthyes, and in the Agnatha and Chondrichthyes than in

Osteichthyes. Marine species of teleosts have a slightly smaller total water content than fresh-water species.

7. Intracellular water was calculated by subtracting extracellular fluid from total body water. The relationship of this compartment to the taxonomic series is almost exactly reversed from that of plasma and extracellular fluid: a relatively small intracellular volume is characteristic of primitive groups and a larger volume of more advanced groups. Marine teleosts have a somewhat smaller intracellular volume than fresh-water teleosts, likewise a reversal of the relation in plasma and extracellular fluid.

8. The phylogenetic and ecological patterns become somewhat more distinct when the volumes are expressed as per cents of total water rather than of body weight.

9. Some of the differences shown are small and statistically questionable, and comparisons involving Agnatha are based on only one species. However, the over-all pattern suggests that, in general, the more advanced forms, as opposed to the primitive, and the fresh-water teleosts, as opposed to the marine teleosts, function with a smaller proportion of mediating fluid and relatively more protoplasmic water. The circulating fluid thus appears to be utilized more effectively in the advanced aquatic vertebrates and, to a lesser degree, in fresh-water than in marine forms.

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